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Prevalence of *Toxoplasma gondii* Antibodies in Muskox (*Ovibos moschatus*) Sera from Northern Canada

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ABSTRACT: Prevalence of antibodies to *Toxoplasma gondii* was determined in 203 muskoxen (*Ovibos moschatus*) from 3 geographically distinct areas of northern Canada (near the hamlets of Kugluktuk and Cambridge Bay, Nunavut and Holman, Northwest Territories) by the modified agglutination test (MAT). Antibodies were found in 13 (6.4%) of 203 animals with MAT titers of 1:25 in 2, 1:50 in 7, 1:200 in 2, 1:400 in 1, and 1:800 in 1. The 4 muskoxen with MAT titers ≥1:200 were adult females and were among 10 animals examined from a mainland population near Kugluktuk. The seroprevalence was lower in Victoria Island muskoxen collected near Cambridge Bay (4.6% of 151) and Holman (4.8% of 42). This is the first serologic survey for *T. gondii* infection in muskoxen.

Toxoplasma gondii occurs worldwide and is prevalent in numerous species of warm-blooded animals (Dubey and Beattie, 1988). Natural infections have been described in at least 19 species of captive and free-ranging ungulates (Ratcliffe and Worth, 1951; Riemann et al., 1974; Gorman et al., 1986; Stover et al., 1990), including moose in Alaska (Zarnke et al., 1997). We report the first serologic survey of *T. gondii* infection in muskoxen (*Ovibos moschatus*).

Muskoxen are found in Canada, Alaska, Greenland, and Scandinavia with approximately 75% of the world's population occurring in northern Canada. They are widely distributed across the Canadian Arctic, where they are the largest herbivore and are preyed on by wolves (*Canis lupus*) and grizzly bears (*Ursus arctos horribilis*). Muskoxen are also an important food for the Inuit in northern Canada.

Sera were collected from a total of 203 muskoxen at 3 locations in the Northwest Territories and Nunavut (Fig. 1). Two collections were done in conjunction with commercial muskox meat harvests on Victoria Island conducted by the Holman and the Ekaluktutiak (Cambridge Bay) Hunters' and Trappers' Associations. The Holman collection was done near Minto Inlet (71°20′N, 117°1′W) from 22 to 25 March 1994. The Cambridge Bay collection occurred near the Ekalluk River by Wellington Bay (69°54′N, 106°38′W) from 7 to 12 March 1998. The third muskox collection was done as part of a treatment trial for the lungworm *Umingmakstrongylus pallikuukensis* (Gunn and Wobeser, 1993; Hoberg et al., 1995) where 10 marked animals were collected in November of 1991 near the hamlet of Kugluktuk (67°54′N, 116°38′W).

Muskoxen were shot by local hunters, and immediately following death, a blood sample was collected from each animal by cutting a jugular vein with a knife and collecting blood directly into 10-ml vacutainer tubes or 80-ml plastic tubes. The blood was kept warm (above 0 C) and transported back to the field camps. Blood was allowed to clot for 12–24 hr, centrifuged for 10 min, and the serum collected using plastic disposable pipets and stored in 2-ml vacutainer tubes. Sera were frozen immediately at outside ambient temperatures (−15–40 C) until transport to the laboratory where they were frozen at −20 C.

Muskox sera were transported frozen in 1998 to the U.S. Department of Agriculture Parasite Biology and Epidemiology Laboratory, Beltsville, Maryland for serologic testing. The modified agglutination test (MAT) was used for measuring antibodies to *T. gondii* as described by Dubey and Desmonts (1987). Sera were screened at 1:25, 1:50, and 1:500 dilutions. Seropositive samples were further tested in 2-fold dilutions (1:25–1:1,600). A MAT titer of 1:25 was considered an indicator of *T. gondii* infection based on studies in pigs (Dubey, Thulliez, and Powell, 1995; Dubey, Thulliez et al., 1995; Dubey, 1997).

Antibodies to *T. gondii* were found in 13 (6.4%) of 203 sera. The antibody titers were 1:800 (1 serum), 1:400 (1 serum), 1:200 (2 sera), 1:50 (7 sera), and 1:25 (2 sera) (Table I). With respect to location, antibodies were found in 7 of 151 (4.6%) animals from Cambridge Bay, in 2 of 42 (4.8%) animals from Holman, and 4 of 10 (40%) animals from Kugluktuk. Seroprevalence in muskoxen on Victoria Island was 9 of 193 (4.7%), and seroprevalence in mainland muskoxen was 4 of 10 (40%). With respect to age, the seroprevalence was 0 of 13 in calves, 2 of 46 (4.3%) in yearlings, 0 of 23 in juveniles, 10 of 100 in adults and 1 of 21 (4.8%) in muskoxen of unknown age. With respect to sex, *T. gondii* antibodies were found in 7 of 108 (6.5%) females and 6 of 92 (6.5%) males.

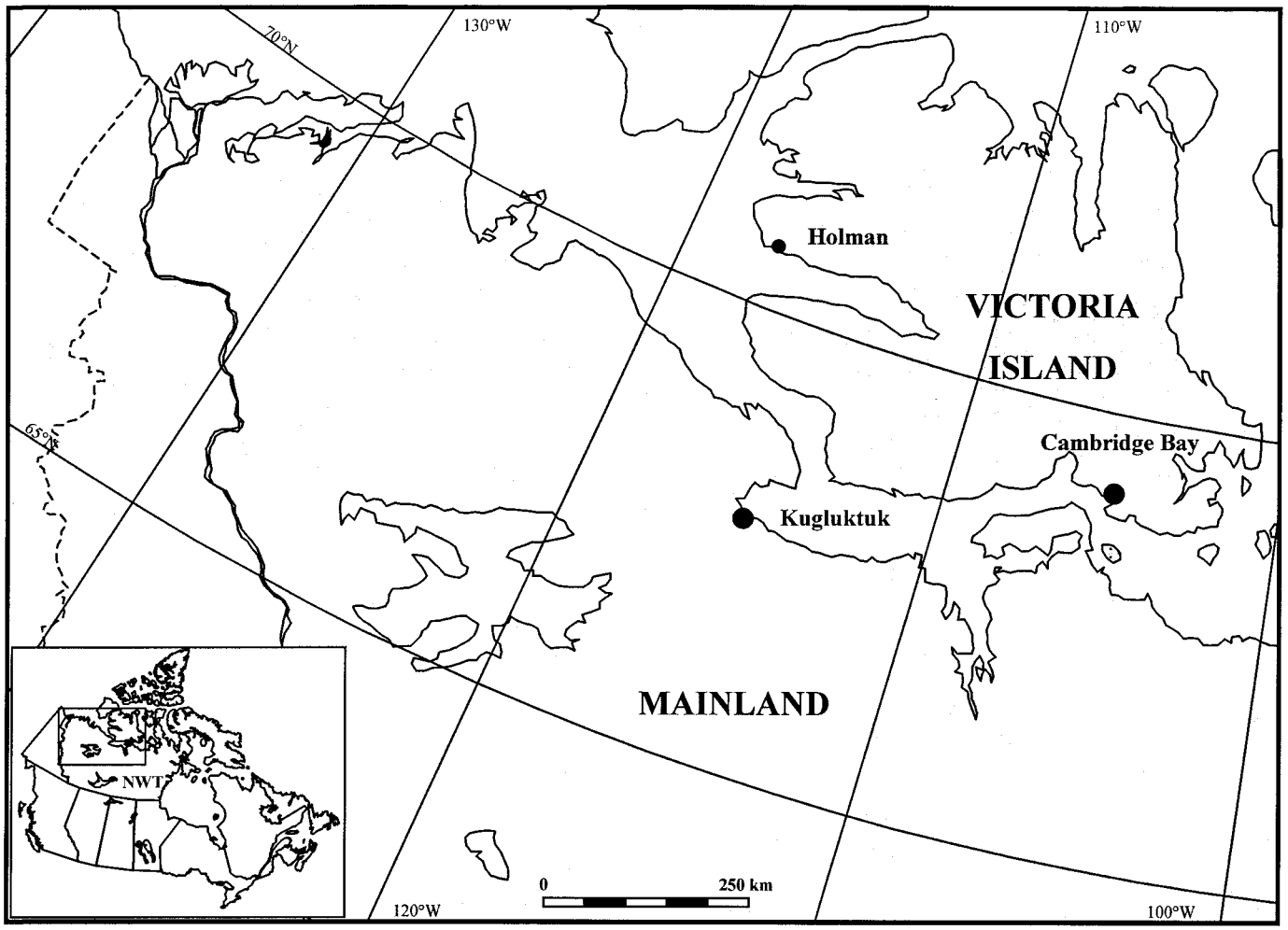

FIGURE 1. Map of Canada and Northwest Territories.

TABLE I. *Toxoplasma gondii* antibodies in sera from naturally exposed, free-ranging muskoxen from northern Canada.

Animal I.D.	Location	Sex	Age (yr)	Antibody titer
1105	Kugluktuk*	F	5	800
1107	Kugluktuk	F	5	400
1103	Kugluktuk	F	4.5	200
1101	Kugluktuk	F	5	200
M019	Holman†	F	Adult	50
M034	Holman	M	Adult	50
98-38	Cambridge Bay‡	F	Unknown	50
98-59	Cambridge Bay	F	4	50
98-191	Cambridge Bay	M	4	50
98-158	Cambridge Ban	M	4	50
98-102	Cambridge Bay	M	4	50
98-148	Cambridge Bay	M	2	25
98-36	Cambridge Bay	M	5	25

* Samples collected November 1991.
† Samples collected March 1994.
‡ Samples collected March 1998.

There is no information on the sensitivity and specificity of the MAT for the diagnosis of toxoplasmosis in muskoxen. Judging from the validation study of the MAT in pigs naturally and experimentally infected with *T. gondii* (Dubey, Thulliez, and Powell, 1995; Dubey, Thulliez et al., 1995; Dubey, 1997), however, a MAT titer of 1:25 is considered specific for toxoplasmosis in pigs.

The finding of no difference in the prevalence of *T. gondii* antibodies between male and female muskoxen is consistent with findings in other free-ranging ungulate species (Mas Bakal et al., 1980; Mohammed and Hussein, 1994; Ferreira et al., 1997), as is the higher prevalence in adult and young-adult animals than in juveniles (Mohammed and Hussein, 1994; Ferreira et al., 1997). These findings, however, must be interpreted with caution as 4 of the positive adult females were from the mainland population where no males or juveniles were sampled. Serological surveys for *T. gondii* in a wide range of wild mammals from 10 study sites across the United States found the prevalence of antibodies was highest in carnivores (52%) and progressively lower in omnivores (21%) and herbivores (9%) (Smith and Frenkel, 1995). The *T. gondii* antibody prevalence in muskoxen in this study (6.4%) was consistent with this pattern.

Domestic and wild Felidae are the only known definitive hosts of *T. gondii* and are required to complete the parasite's enteroepithelial life cycle and shed oocysts (Dubey and Beattie, 1988). The only wild felid found in northern Canada is the Canadian lynx (*Lynx canadensis*), and it has been shown to shed *T. gondii* oocysts (Aramini et al., 1998). Lynx may overlap in range with the mainland muskoxen tested near Kugluktuk (40% antibody prevalence), but lynx sightings are unusual as the muskoxen are distributed year-round on the tundra above the treeline (Gunn and Fournier, 1999), and lynx generally remain below the treeline. On Victoria Island where the Cambridge Bay and Holman samples were collected (4.7% antibody prevalence) lynx sightings are extremely rare. Domestic cats (*Felis domesticus*) are uncommon in these communities, and it is unlikely that feral domestic cats would survive outside of the communities. Given the absence of wild felids on Victoria Island, the source of oocyst production and exposure for the muskoxen is unclear. Although caribou (*Rangifer tarandus*) have a seasonal migration between Victoria Island and the nearby mainland, muskox movements are unusual and irregular (Gunn et al., 1997).

In many species, infection with *T. gondii* may be common, but clinical disease is rare (Garell, 1999). Clinical signs of toxoplasmosis can be nonspecific and vary depending on the organ systems affected. *Toxoplasma gondii* is also an important cause of abortion and stillbirth in sheep and goats (Stover et al., 1990; Dubey and Beattie, 1998). It is interesting to note that the 4 muskoxen that had high antibody titers (1:200–1:800) were adult females sampled early in the gestational period, November 1991, near Kugluktuk. This mainland muskox population declined significantly between the 1980s and the most recent survey in 1994 (Fournier and Gunn, 1998). Reasons for the apparently low pregnancy rates that contributed to the decline (Gunn and Fournier, 2000) are unknown, but the potential role of diseases or parasites such as *T. gondii* in this decline should be considered. The epidemiology and clinical significance of toxoplasmosis in free-ranging muskox populations need to be investigated further.

Resource harvesting is important to the Northwest Territories (NWT) economy, with many northern people still relying on hunting and fishing for food and income. Muskoxen are currently harvested for subsistence use, commercial meat sales, and big game hunting. The replacement cost of the 5 million kg of meat and fish harvested domestically each year is estimated at U.S. $40 million, with the estimated value of muskox harvesting at $3 million and increasing (Gunn et al., 1991). In 1988, about 22% of the 52,300 people in the NWT and Nunavut resided in the 20 communities that had access to muskox harvesting. Any factors that affect the health of muskoxen could reduce the sustainability of the muskox harvest and decrease the availability of this food and income source to local people.

Toxoplasma gondii is an important zoonotic agent, and infections in muskoxen may be of public health significance. In Alaska, 28% of 1,572 aboriginal people tested during the early 1970s had antibodies to *T. gondii* (Peterson et al., 1974). Transmission can occur through the ingestion of tissue cysts in undercooked muscle, liver, or other tissues, or ingestion of oocysts from the feces of domestic or wild felids. In order to reduce the risk of exposure, meat and other tissues from infected animals should be cooked to an internal temperature of 70 C before human consumption (Dubey and Beattie, 1998).

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Growth of the Genital Primordium as a Marker to Describe a Time Course for the Heterogonic Larval Development in *Strongyloides stercoralis*

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ABSTRACT: A time course for the heterogonic development of *Strongyloides stercoralis* is described and a method for distinguishing the early larval stages of this nematode is proposed. The number of cells in the developing gonad were counted at various time intervals of incubation, along with the percentage of larvae in molt at each interval. The time course of growth of the gonad follows a pattern comparable to that reported for body length in an idealized general nematode. A model for the heterogonic development of *S. stercoralis* is proposed, which, although similar to other nematode developmental models, is stage specific for *S. stercoralis*, allowing the otherwise morphologically similar rhabditiform stages (L1, L2) to be distinguished.

The nematode, *Strongyloides stercoralis*, may develop through either of 2 different life histories. These are the direct, or homogonic, pathway and the indirect, or heterogonic, pathway. In the former, worms develop after hatching through the first (L1) and second (L2) to the third (L3) larval stage. The latter are infective and can seek a new host or reinfect their original definitive host. In contrast, heterogonic worms develop through 4 rhabditiform larval stages into free-living, reproducing adults, which in turn produce a new generation of free-living larvae. These develop to the L3, which is infective (Hawdon and Schad, 1991).

The morphological differences between heterogonic L1 and L2 stage larvae are subtle; L2 is just slightly larger than L1. Therefore, it has been difficult to distinguish these larvae. Presumably because of this, a time course for the early stages of the heterogonic pathway has not been described. However, the developing gonad is an effective marker for distinguishing the early larval stages in this nematode. The genital primordium is a small organ that grows progressively and eventually differentiates into the worm's reproductive structures. By counting the number of cells in this primordial organ as the larvae develop and recording the percentage of worms in molt at each time interval, we arrived at a time course for heterogonic development.

Strongyloides stercoralis L1-stage larvae were taken from the feces of an infected dog by use of the Baermann method. The larvae were mounted on agar pads, which were prepared from 2 ml of 1.5% low melting point agarose (Type 1-A, Sigma Chemical Co., St. Louis, Missouri) containing 6μl 1-phenoxy-2-propanol (Janssen Chimica, Geel, Belgium), which serves as an anesthetic (Ashton, et al., 1998). The anesthetized larvae were viewed at ×940 by differential interference microscopy, which made the individual cells in the primordial gonad visible and distinguishable.

Photographs were taken every 2 μm, focusing through the diameter of the developing gonad. These photographs recorded the number of cells in the genital primordium of larvae freshly extracted from feces. This procedure was repeated hourly using larvae that had been cultured for 1 through 9 hr. Larvae were cultured in 15 × 60–mm plastic petri dishes with 3 ml of 1.5% Bacto-Agar (Difco Laboratories, Detroit, Michigan), seeded with approximately 1 μl of parasite-free feces, and placed in

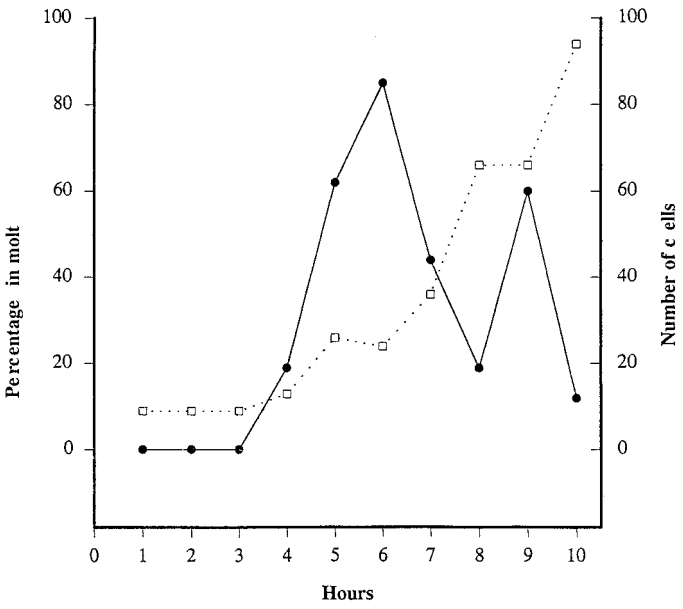

FIGURE 1. Time course for the development of the genital rudiment of *S. stercoralis* in relation to larval molting. □ Number of cells in the genital rudiment. ● Percentage of worms molting, i.e., showing a separation of the cuticle.